PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
V. Sandig, et al.	Group Art Unit: 1633
Application No.: 10/578,043	Examiner: Leavitt, M.G.
Filed: January 9, 2008	Confirmation No.: 5415
For: Immortalized Avian Cell Lines for Virus Production	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

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I, Ingo Jordan, hereby declare:

1. I hold a Ph.D. degree in biochemistry and retrovirology and consider myself one of skill in the art of virology.

2. I have published 13 articles indexed in the PubMed NCBI library.

3. I have reviewed the claims of U.S. Application No. 10/578,043, and I have been informed that these claims are rejected on the basis of obviousness, that one of skill in the art would have recognized that either or both adenoviral E1A gene and E1B genes are sufficient to immortalize avian cells.

4. I disagree with the Office Action's assertion. I am of the view that complete immortalization of avian cells with E1A and E1B 55K from mastadenoviruses is an unexpected result.

5. The problem to be solved by the inventors was the provision of a cell line suitable for the production of vaccines or gene therapy vectors. During replication of vaccines, the producer cell line disintegrates. Components derived from the host (nucleic acids that still may have coding potential and active proteins) may, thus, be co-purified with the vaccines. Such impurities cannot be fully avoided and the risk emanating from such impurities has to be calculated by multiplying danger associated with a component and degree of depletion obtained in the purification process. For this reason, the level of knowledge about the producer cell line must be exhaustive and as complete as possible. Introduction of dangerous factors and induction of unknown changes such as uncontrolled mutation events to the cellular genome should be avoided.

6. The inventors provided an immortal avian cell line with a known genetical and biochemical background. They surprisingly found that the expression of two defined viral genes, namely (i) a first viral gene which is a Mastadenovirus early region 1A (E1A) gene, and (ii) a second viral gene which codes for a Mastadenovirus early region 1B 55K (E1B 55K) protein in the avian cell line is necessary and sufficient for the immortalization of said cell line. Spontaneous transformation events were not observed by the inventors in this cell line (see page 33, second paragraph of the description of the present patent application). Accordingly, this cell line is ideally suited for the production of vaccines and gene therapy vectors.

7. Immortalizing factors from SV40 or high risk human papillomavirus HPV-16 efficiently transform cells. However, due to this transformation efficiency, they are considered as being too dangerous under experts for use in a vaccine preparation. The tumorigenicity of SV40 large T antigen, for example, represents a critical factor in the preparation of vaccine compositions which usually contain more or less contaminations caused by the known or unknown (in spontaneously immortalized cells) mediators or reagents used for immortalization of producer cells (see www.fda.gov/ohrms/dockets/ac/08/briefing/2008-4384B1_3.pdf). The assertion in Bouquet et al. (US 6,255,108 B1) that a vector comprising SV40 is not oncogenic (see column 1, lines 16 to 20) and that cells comprising said vector remain untransformed (see claim 1 and column 2, lines 4 and 5) stands in striking contrast to the established knowledge in the field of virology.

8. The E1A gene is derived from a virus not associated with human tumors. However, because of its gentle immortalizing activity it must be supplemented by other events within the cell. Guilhot et al. "THE 12S ADENOVIRAL E1A PROTEIN IMMORTALIZES AVIAN CELLS AND INTERACTS WITH THE AVIAN RB PRODUCT", Oncogene, 1993, 619-624 refers to the introduction of the gene encoding 12S adenoviral E1A protein in quail cells. The 12S protein is a splice-variant of full-length E1A (also designated as 13S). It differs from the 13S protein in that the conserved region 3 (CR3) region is missing. Both proteins, 12S and 13S, are encoded by the E1A nucleic acid sequence comprised in the cell of claim 1. Although the 12S protein transforms with a higher efficiency than the 13S protein, complete immortalization with 12S has not been described in Guilhot et al. From the teaching of Guilhot et al., it is clear that the 12S adenoviral E1A protein is important for the immortalization of avian cells - by affecting the function of the retinoblastoma (Rb) protein - but not sufficient. It is particularly mentioned in Guilhot et al., 1993 that the isolated cell lines were all obtained after a growth crisis period, which suggests that other events are important for immortalization. The E1A 12S gene expression is rather considered in Guilhot et al., 1993 as a first event inducing uncontrolled cell proliferation that would predispose some cells to mutations or rearrangements in regulatory genes, thereby enabling immortalization (see page 623, left column, third paragraph). Such an approach prohibitively confounds calculation and definition of risks posed by vaccine preparations derived from such cells for several reasons: (1) The E1A 12S gene is not balanced by the 13S version which appears to be required to fine-tune the reprogramming of the epigenetic code in the transfected cell; and (2) the unknown secondary event induced by the retroviral E1A 12S gene may by itself be sufficient for transformation, i.e. a factor may have been selected for that is similarly or even more aggressive as an SV40-derived gene.

9. As discussed in the previous declaration, human adenoviruses cannot replicate in avian cells. However, the inventors noticed that human adenoviruses can successfully enter avian cells. Thus, the lack of replication is not due to the fact that said adenoviruses do not find the correct receptors for viral entry. It is predicted that the viral regulatory pathways within the avian cell, which include E1A and E1B 55K, appear to be dysfunctional when compared to mammalian cells, particularly human cells. Indeed, there are no homologous genes to E1A and E1B in avian adenoviruses. Generally, viruses within a family tend to conserve the sequence of regulatory proteins. However, this is different between mastadenoviruses and fowl adenoviruses. According to Cao et al. "SEQUENCE

AND TRANSCRIPTIONAL ANALYSIS OF TERMINAL REGIONS OF THE FOWL ADENOVIRUS TYPE 8 GENOME", 1998, 79, pages 2507 to 2516, no mastadenovirus homologues of E1A and E1B could be found in fowl. It is mentioned in Cao et al. that completely unrelated proteins regulate virus-host interaction although otherwise the fowl adenoviruses use the same or very similar strategies to initiate DNA replication and DNA packaging (see abstract and introductory part). In addition, the mechanism of inactivation of p53 (a target of E1B 55K) appears to differ in mammalian and chicken cells as discussed in Kim et al. "ALTERATIONS IN P53 AND E2F-1 FUNCTION COMMON TO IMMORTALIZED CHICKEN EMBRYO FIBROBLASTS" ONCOGENE, BASINGSTOKE, HANTS, GB, vol. 20, no. 21, 2001, pages 2671-2682 (see page 2677, left column, second paragraph). The extremely low homology between chicken and human p53, which we have brought to the Examiner's attention in our last declaration, is also attributable to the different mechanism in mammals and birds for regulation of p53 activity. Based on the above, the circumstance that the 12S protein of E1A from human adenovirus is able to immortalize avian cells together with other undefined mutation events cannot be indicative of the function of the E1A and/or the E1B 55K protein in avian cells considering that there exists no E1A and no E1B homolog to human adenoviruses in avian adenoviruses.

10. E1A is only a safe and gentle immortalizing factor in a primary avian cell if it is balanced by an activity that targets p53. However, to our knowledge the literature and the general opinion in the art suggest that E1B is not such a factor in avian cells and should not help to yield a healthy and suitable cell line. Accordingly, the skilled person considering the prior art documents cited, namely Guilhot et al., Kim et al., Bouquet et al. (US 6,255,108 B1), and Pau et al. (US 7,192,759 B1), could not expect to immortalize avian cells with the expression of (i) a first viral gene which is a Mastadenovirus early region 1A (E1A) gene, and (ii) a second viral gene which codes for a Mastadenovirus early region 1B 55K (E1B 55K) protein in said cells.

11. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. All of the statements I made herein are made of my own volition. I understand that willful false statements may subject me to fines, imprisonment or both, pursuant to Section 1001 of Title 18 of the United States Code.

Signed: _____

Date: January 27, 2012

9